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Comparison of the pharmacodynamics of protamine zinc insulin and insulin degludec and validation of the continuous glucose monitoring system iPro2 in healthy cats

Salesov, Elena ; Zini, Eric ; Riederer, A ; Lutz, Thomas A ; Reusch, Claudia E

Abstract: With the aim to improve current therapeutic and monitoring options for diabetic cats, the present study compared pharmacodynamic parameters of protamine zinc insulin (PZI) and insulin degludec and validated the continuous glucose monitoring system (CGMS) iPro2 with Sof-sensor and Enlite-sensor focusing on the low glycemic range. Three doses (0.1, 0.2 and 0.3IU/kg) of the two insulin preparations and the CGMS iPro2 with two different sensors were tested in six healthy cats. After each insulin administration, onset of action, time to glucose nadir and duration of action were calculated by measuring glucose concentrations with a portable blood glucose meter (PBGM). After sensor placement, paired PBGM and sensor glucose measurements were done and analytical and clinical accuracy were calculated according to the ISO 15197:2013 criteria. Onset of action, time to glucose nadir and glucose nadir were similar for both insulin formulations. Duration of action of insulin degludec was significantly longer than those of PZI at 0.1IU/kg ($P=0.043$) and 0.2IU/kg ($P=0.043$). Overall, 166/191 (87%) Sof-sensor measurements and 106/121 (88%) Enlite-sensor measurements met ISO criteria for analytical accuracy, and all sensor measurements fulfilled ISO criteria for clinical accuracy. Insulin degludec was well tolerated in healthy cats and showed longer duration of action than PZI. Further studies on the use of insulin degludec in diabetic cats might be recommended. Both sensors had good clinical accuracy, when used with the CGMS iPro2, but the analytical accuracy was below the minimum set by ISO 15197:2013.

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1 **Comparison of the pharmacodynamics of protamine zinc insulin and**
2 **insulin degludec and validation of the continuous glucose monitoring**
3 **system iPro2 in healthy cats**

4
5 Elena Salesov¹, Eric Zini¹, Angelina Riederer¹, Thomas A Lutz², Claudia E
6 Reusch¹

7
8 ¹Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University
9 of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland

10 ²Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich,
11 Winterthurerstrasse 260, 8057 Zurich, Switzerland

12
13
14 **Corresponding author:**

15 Elena Salesov, DVM

16 Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of
17 Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland; E-mail:

18 esalesov@vetclinics.uzh.ch

19 **Abstract**

20 With the aim to improve current therapeutic and monitoring options for
21 diabetic cats, the present study compared pharmacodynamic parameters of
22 protamine zinc insulin (PZI) and insulin degludec and validated the
23 continuous glucose monitoring system (CGMS) iPro2 with Sof-sensor and
24 Enlite-sensor focusing on the low glycemic range.

25 Three doses (0.1, 0.2 and 0.3 IU/kg) of the two insulin preparations and the
26 CGMS iPro2 with two different sensors were tested in six healthy cats.

27 After each insulin administration, onset of action, time to glucose nadir and
28 duration of action were calculated by measuring glucose concentrations
29 with a portable blood glucose meter (PBGM). After sensor placement,
30 paired PBGM and sensor glucose measurements were done and analytical
31 and clinical accuracy were calculated according to the ISO 15197:2013
32 criteria.

33 Onset of action, time to glucose nadir and glucose nadir were similar for
34 both insulin formulations. Duration of action of insulin degludec was
35 significantly longer than those of PZI at 0.1 IU/kg ($P = 0.043$) and 0.2 IU/kg
36 ($P = 0.043$). Overall, 166/191 (87%) Sof-sensor measurements and 106/121
37 (88%) Enlite-sensor measurements met ISO criteria for analytical accuracy,
38 and all sensor measurements fulfilled ISO criteria for clinical accuracy.

39 Insulin degludec was well tolerated in healthy cats and showed longer
40 duration of action than PZI. Further studies on the use of insulin degludec in
41 diabetic cats might be recommended. Both sensors had good clinical

42 accuracy, when used with the CGMS iPro2, but the analytical accuracy was
43 below the minimum set by ISO 15197:2013.
44

45 **Keywords**

46 Protamine zinc insulin; insulin degludec; iPro2; Sof-sensor; Enlite-sensor;
47 continuous glucose monitoring system

48

49 **Abbreviations**

50 CGMS continuous glucose monitoring system

51 DM diabetes mellitus

52 ISO international organization for standardization

53 PBGM portable blood glucose meter

54 PZI protamine zinc insulin

55 **1. Introduction**

56 Insulin is the cornerstone of treatment of cats with diabetes mellitus (DM).
57 Duration of insulin action was reported to be the most important factor, that
58 influences choice of insulin (Smith et al., 2012). A protamine zinc
59 preparation (PZI) for use in cats recently became available on the European
60 market. It is an intermediate to long-acting recombinant human insulin that
61 has been shown to be effective in diabetic cats (Nelson et al., 2009;
62 Norsworthy et al., 2009; Ward et al., 2015), but information about its
63 pharmacodynamics in cats is scarce. Insulin degludec is a new ultra-long
64 acting human insulin analogue. The formulation with a concentration of 100
65 IU/mL is available on the market and could be used in diabetic cats.
66 Compared with insulin glargine, insulin degludec has a predictable and
67 stable glucose-lowering effect with fewer episodes of hypoglycemia in
68 human patients with type 1 or type 2 DM (Birkeland et al., 2011; Heller et
69 al., 2012; Rodbard et al., 2013). Moreover, when given three times a week,
70 insulin degludec provides glycemic control comparable to once-daily
71 treatment with insulin glargine (Zinman et al., 2011). To our knowledge,
72 there are no reports of the glucose-lowering effects of insulin degludec in
73 cats.
74 The generation of glucose curves is commonly used by veterinarians to
75 evaluate feline diabetic control (Smith et al., 2012). It helps in the
76 identification of hypoglycemia, and supports the decisions on treatment
77 adjustments. However, glucose curves have some limitations, even if the
78 glucose measurements are obtained in the cat's home environment. They do

79 not provide continuous information about blood glucose concentrations, or
80 the glucose nadir and the glucose peak could be missed. In addition, the
81 duration of action of the insulin cannot be determined if home monitoring is
82 limited to a short period of time (i.e., <12 h). Another important limitation is
83 that not all owners are able to collect blood from their cats and generate
84 blood glucose curves.

85 Real-time continuous glucose monitoring systems (CGMS) continuously
86 measure the glucose concentration in the subcutaneous fat via a sensor
87 containing glucose oxidase and immediately display recorded values on a
88 monitor. These systems are considered useful for monitoring cats with DM
89 (Ristic et al., 2005; Moretti et al., 2010; Dietiker-Moretti et al., 2011; Gough
90 et. al., 2013; Hafner et al., 2013; Surman and Fleeman, 2013). However,
91 CGMSs are not suitable for home-monitoring because the maximum
92 distance between the cat and monitor should be only a few meters (Dietiker-
93 Moretti et al., 2011; Hafner et al., 2013). The CGMS iPro2 was designed to
94 measure and record glucose values in humans for up to 7 days without
95 displaying the data on a monitor; instead, at the end of the monitoring
96 period, the data are uploaded on a computer and evaluated retrospectively.
97 Because the iPro2 does not involve a monitor, it may be suitable for use in
98 diabetic cats in their home environment. Two different sensor types are
99 available, the enhanced Enlite-sensor and the Sof-sensor; the former is
100 shorter, more flexible and more accurate than the latter (Siegmond et al.,
101 2011). In human patients, the Enlite-sensor tends to measure glucose levels
102 lower than the reference over the entire glucose range, whereas the Sof-

103 sensor measurements tend to be higher than reference values in the
104 hypoglycemic range and lower than reference values in the hyperglycemic
105 range (Calhoun et al., 2013). The use of the CGMS iPro2 and its reliability
106 using these two sensors have not been described in cats.
107 The aims of the study were to compare pharmacodynamic parameters of
108 PZI and insulin degludec in cats, including onset of action, time to glucose
109 nadir, glucose nadir and duration of action. Furthermore, ease of use,
110 tolerability, side effects, reliability, and the accuracy of CGMS iPro2 using
111 the two different sensors were evaluated. Particular attention was paid to the
112 accuracy of the iPro2 in the low glycemic range, because reliability of
113 measurements is crucial in hypoglycemic cats.

114 **2. Materials and Methods**

115 **2.1 Animals**

116 Six healthy purpose-bred, neutered male, domestic shorthair cats were used.
117 The median age was 3.7 years (range 3.4-3.7) and median body weight was
118 5.0 kg (range 4.7-5.9). All cats had body condition score of 5 on a 9-point
119 scale. They were housed in groups of two, and were fed a commercial dry
120 food for adult cats twice daily. Food intake was adjusted to maintain a stable
121 body weight. During the 24 h before and after insulin administration, the
122 cats were individually kept in cages routinely used for hospitalized animals.
123 Food was withheld for 10 h before and 24 h after insulin injection. Cats had
124 free access to water. The study protocol was approved by the veterinary
125 office of the canton Zurich (permission number: 110/2014).

126

127 **2.2 Evaluation of two insulin preparations**

128 PZI 40 IU/mL (ProZinc, Boehringer Ingelheim, Basel, Switzerland) and
129 insulin degludec 100 IU/mL (Tresiba, Novo Nordisk Pharma, Küsnacht ZH,
130 Switzerland) were tested in a randomized crossover trial. Each cat received
131 0.1, 0.2 and 0.3 IU/kg of PZI and insulin degludec SC, respectively, two
132 weeks apart. Insulin syringes with 0.5 IU markings (Omnican 20, U-40
133 insulin, BBraun, Melsungen, Germany; BD MicroFine 0.3 ml, U-100
134 insulin, BD Medical, Le Pont de Claix, France) were used. The dose was
135 rounded up to the nearest half unit. Capillary blood glucose was measured at
136 the inner pinna of an ear with a portable blood glucose meter (PBGM)
137 AlphaTRAK2 (Abbott Animal Health, Baar, Switzerland) 30 and 5 minutes

138 before and 30, 60, 90, 120, 180, 240, 300 and 360 minutes after insulin
139 injection and then every 2 h for another 18 h. Biochemical hypoglycemia
140 was defined as blood glucose <3.6 mmol/L.
141 If hypoglycemia caused vocalization, vomiting, tremors, or seizures, cats
142 received canned food with glucose syrup (Jubin®, Andreas Jubin Pharma,
143 Bochum, Germany), or 50% glucose solution (0.5-1 mL/kg) was infused
144 intravenously. Hypoglycemia was not corrected, if only reduced physical
145 activity was observed.
146 Onset of insulin action, time to glucose nadir, and duration of insulin action
147 were calculated as described by Clark et al. (2012). The onset of insulin
148 action was defined as the interval between insulin administration and the
149 first glucose concentration that was at least 2 standard deviations lower than
150 baseline. The time to glucose nadir was defined as the interval between
151 insulin administration and the lowest measured glucose concentration. The
152 duration of insulin action was defined as the interval between the onset and
153 the end of insulin action, when measured glucose concentrations had
154 returned to within 2 standard deviations of baseline. The glucose data were
155 withdrawn from the calculation, if the insulin tests were terminated
156 (feeding, infusion of glucose solution), or if the onset of action could not be
157 achieved.

158

159 **2.3 Technical features of the CGMS iPro2 and the two sensors**

160 The CGMS iPro2 (Medtronic, Münchenbuchsee, Switzerland) consists of a
161 digital recorder (Fig.1a) and a disposable sensor. The Sof-sensor

162 (Medtronic, Münchenbuchsee, Switzerland) is 14.7 mm long and has a
 163 volume of 3.6 mm³ (Fig. 1b). It is inserted through the skin into the
 164 subcutaneous fat by means of a 17 mm 22 G needle at an angle of 45-60°
 165 and is fully hydrated within 10-15 minutes after positioning. The enhanced
 166 Enlite-sensor (Medtronic, Münchenbuchsee, Switzerland) is 9.6 mm long
 167 and has a volume of 0.2 mm³ (Fig. 1c). It is inserted by means of a 10.5 mm
 168 27 G needle at a 90° angle and is fully hydrated within 5 minutes after
 169 positioning. Both sensors measure the interstitial glucose concentration in
 170 the subcutaneous fat via an electrode that contains glucose oxidase (Hafner
 171 et al., 2013). The iPro2 measures glucose concentrations between 2.2 and 22
 172 mmol/L every 5 minutes for up to 7 days. Data analysis is done using the
 173 CareLink iPro software (Medtronic, Münchenbuchsee, Switzerland).

174

175 **2.4 Evaluation of CGMS with two sensors**

176 The cats were alternately implanted with a Sof-sensor or an Enlite-sensor 24
 177 h before the insulin injection. The insertion and removal of the sensors were
 178 performed without sedation. Each sensor was placed in the subcutaneous
 179 tissue of the neck area on the right side and secured with cyanoacrylate
 180 adhesive (Cyanolit universal classic, 3M Consumer Healthcare, Rüschlikon,
 181 Switzerland) as described previously (Hafner et al., 2013). The recorder was
 182 connected to the sensor, initialized and then secured using a 3×7 cm piece of
 183 adhesive tape (Fig. 1d). The video in the E-book chapter (Rand and Gottlieb,
 184 2017) contains detailed information about placement of the glucose sensor
 185 and iPro2-recorder. Calibrations were achieved by measuring capillary

186 blood glucose concentrations 1 and 3 h after insertion of the sensor and then
187 every 8-12 h. Blood glucose concentrations were determined by use of the
188 PBGM AlphaTRAK2 previously evaluated for use in diabetic cats
189 (Cohenen et al., 2009; Zini et al., 2009). Each cat carried the sensor and the
190 recorder on it for seven days. Thereafter, they were gently removed from the
191 skin, and the glucose curve was downloaded. Ease of use of the sensors,
192 tolerability, reliability of the measurements and side effects were recorded.

193

194 **2.5 Statistical analysis**

195 Results are reported as median and range or as percentage, as appropriate.
196 Differences in pharmacodynamic parameters (onset of insulin action, time
197 to glucose nadir, glucose nadir and duration of insulin action) between the
198 same doses of two insulin preparations were analyzed using the Wilcoxon
199 matched pairs signet rank test. The Friedman test was used to compare three
200 different doses of one insulin formulation. Analytical and clinical accuracy
201 of the sensors was calculated conforming to the international organization
202 for standardization (ISO) 15197:2013 using Bland and Altman diagrams
203 and consensus error grid coordinates, respectively (Bland and Altman,
204 1986; Clarke et al., 1987). Accordingly, to be considered accurate, at least
205 95% of glucose readings had to fall within ± 0.83 mmol/L of those
206 measured with the reference method for concentrations <5.5 mmol/L or
207 within $\pm 15\%$ for concentrations ≥ 5.5 mmol/L, and at least 99% of readings
208 had to be in zones A and B of the consensus error grid. To address the
209 clinical importance of hypoglycemia, glucose curves with concentrations

210 predominantly <3.6 mmol/L were chosen for analysis. Paired glucose
211 readings from the PBGM and the two sensors were divided into a group
212 with PBGM readings <5.5 mmol/L and a group with PBGM readings ≥ 5.5
213 mmol/L. Additionally, Spearman correlation coefficients (ρ) were
214 calculated for paired glucose readings. Differences were considered
215 significant at $P < 0.05$. A commercial software (GraphPad PRISM 6,
216 GraphPad Software, La Jolla, USA) was used for analysis.

217 **3. Results**

218 **3.1 Evaluation of PZI and insulin degludec**

219 Protamine zinc insulin and insulin degludec were well tolerated by all cats
220 and there were no injection site reactions. If the glucose lowering effect
221 could be achieved, all three doses of both insulin formulations caused
222 transient mild weakness, that was associated with biochemical
223 hypoglycemia and resolved without treatment within 1-3 h in all cats. The
224 onset of insulin action could not be achieved in three different cats treated
225 with 0.1 IU/kg of PZI (one cat), 0.1 IU/kg of insulin degludec (one cat), and
226 0.2 IU/kg of PZI (one cat). At the highest dose (0.3 IU/kg), PZI caused
227 hypoglycemia, and vomiting in 3/6 (50%) cats; one of these cats had the
228 same reaction after 0.3 IU/kg of insulin degludec. The hypoglycemia was
229 corrected by feeding in three cats, and the glucose data were withdrawn
230 from the calculation. One cat treated with 0.3 IU/kg of PZI quickly
231 recovered without any interventions.

232 Medians and ranges of pharmacodynamic parameters of both insulin
233 formulations are shown in Table 1. Blood glucose curves are presented in
234 Fig. 2 (a-f). Equal doses of PZI and insulin degludec did not differ with
235 respect to glucose nadir, onset of action, and time to glucose nadir. Median
236 duration of action was significantly shorter for 0.1 IU/kg of PZI (7 h; range
237 1.5-7) than for 0.1 IU/kg of insulin degludec (11 h; range 9-22.5) ($P=0.043$)
238 and for 0.2 IU/kg of PZI (6.8 h; range 4.5-10.5) than for 0.2 IU/kg of insulin
239 degludec (12.5 h; range 8.0-20.0) ($P=0.043$). However, there were no

240 significant differences in the duration of action between two insulin
241 formulations at 0.3 IU/kg.
242 The duration of action of PZI never reached 12 h, while that of insulin
243 degludec was ≥ 12 h in two cats with 0.1 IU/kg and in three cats with 0.2 and
244 0.3 IU/kg (data not shown). Glucose nadir, onset of insulin action, time to
245 glucose nadir, and duration of insulin action of PZI and insulin degludec
246 were similar between different doses of each insulin formulations.

247

248 **3.2 Evaluation of CGMS with the Sof-sensor and Enlite-sensor**

249 The use of a total of 36 sensors (18 Sof and 18 Enlite) was scheduled.
250 However, we used 48 sensors (24 Sof and 24 Enlite), because some sensors
251 had to be replaced. Twenty of twenty-four (83%) Sof-sensors and 17/24
252 (71%) Enlite-sensors were properly placed and initialized. The remaining 11
253 sensors failed because of faulty manufacturing (1 Sof and 2 Enlite) or lack
254 of initialization (3 Sof and 4 Enlite). One Enlite-sensor was bent during
255 insertion. Both sensor types were easy to place and well tolerated by all
256 cats. Abnormal behavior related to the sensors did not occur. All cats had
257 mild local erythema after sensor removal, which resolved spontaneously
258 within 12-24 h. One cat had a small dry scratch wound in the skin at the
259 caudal border of the Sof-sensor and another cat had a similar wound at the
260 caudal border of the Enlite-sensor. Both healed without treatment within 7-
261 10 days after sensor removal.
262 Glucose concentrations were recorded by all 20 properly placed and
263 initialized Sof-sensors and by 13/17 (76%) Enlite-sensors. Four (24%)

264 Enlite-sensors failed to record glucose concentration, because the recorder
265 malfunctioned (two cats), the cat removed the sensor 1 h after insertion or
266 for unknown reasons (one cat). Seventeen of the twenty (85%) functioning
267 Sof-sensors and 6/13 (46%) functioning Enlite-sensors recorded the glucose
268 concentration during the entire 7-day study period. The remaining 3/20
269 (15%) functioning Sof-sensors and 3/13 (23%) Enlite-sensors recorded
270 glucose concentrations during at least 50% of the study period. The four
271 (31%) other functioning Enlite-sensors recorded glucose concentration
272 during <50% of the study period.

273 Uninterrupted recording of glucose concentrations occurred in 8/20 (40%)
274 functioning Sof-sensors and in 8/13 (66%) functioning Enlite-sensors.

275 Overall, there were 27 and 23 interruptions with the Sof-sensor and the
276 Enlite-sensor, respectively. Twenty-three of the twenty-seven (85%)
277 interruptions that occurred with the Sof-sensor and 16/23 (70%) that
278 occurred with the Enlite-sensor lasted <1 h. The remaining four (15%)
279 interruptions with the Sof-sensor and seven (30%) with the Enlite-sensor
280 lasted between 1 and 24 h.

281 A total of 191 paired PBGM-Sof-sensor and 121 paired PBGM-Enlite-
282 sensor glucose measurements were analyzed. One hundred and seventy-
283 seven of one hundred and ninety-one (93%) Sof-sensor measurements and
284 113/121 (93%) Enlite-sensor measurements were between 2.2 and 5.5
285 mmol/L. The remaining measurements were equal to 5.5 mmol/L or in the
286 range between 5.5 and 22 mmol/L. The differences between glucose
287 measurements from the PBGM and the two CGMS iPro2 sensors are shown

288 in Fig. 3. Considering the paired glucose measurements <5.5 mmol/L,
289 160/177 (90%) Sof-sensor measurements and 102/113 (90%) Enlite-sensor
290 measurements were within ± 0.83 mmol/L of the reference method and met
291 the ISO accuracy criteria. Of the paired glucose readings ≥ 5.5 mmol/L, 6/14
292 (43%) of Sof-sensor measurements and 4/8 (50%) Enlite-sensor
293 measurements were within $\pm 15\%$ of the reference method and met the ISO
294 accuracy criteria. Overall, 166/191 (87%) Sof-sensor measurements and
295 106/121 (88%) Enlite-sensor measurements met the ISO criteria for
296 analytical accuracy. All glucose concentrations measured with both sensors
297 were in zone A or B of the consensus error grid coordinates and met the ISO
298 criteria for clinical accuracy (Fig. 4). There were moderate positive
299 correlations between paired measurements from the PBGM and the Sof-
300 sensor ($\rho=0.67$, $P<0.0001$) and between paired measurements from the
301 PBGM and the Enlite-sensor ($\rho=0.69$, $P<0.0001$).

302 **4. Discussion**

303 This study compared pharmacodynamics of PZI and insulin degludec in
304 healthy cats. Marked biochemical hypoglycemia and weakness occurred
305 with both insulin formulations in all tests, when the glucose lowering effect
306 was achieved. Vomiting was limited to three cats receiving the highest dose
307 of PZI and to one cat receiving the highest dose of insulin degludec.

308 Both types of insulin had a similar onset of action, time to glucose nadir and
309 glucose nadir. Median duration of action was significantly longer for insulin
310 degludec than for PZI at 0.1 and 0.2 IU/kg. The differences between two
311 insulins at 0.3 IU/kg was not significant. However, the duration of action
312 was about 1.5 times as long for insulin degludec than for PZI at all dose
313 levels. The duration of action of PZI was shorter than 12 h in all cats
314 irrespective of the doses. This differed from results of a recent study, in
315 which PZI had a duration of action exceeding 12 h in some of the treated
316 diabetic cats, and was therefore considered potentially useful for once-a-day
317 treatment (Ward and Louviere, 2015). Longer duration of action of PZI in
318 the mentioned study could be explained by the applied insulin dose (0.5
319 IU/kg or higher). In our study, higher insulin dose was not associated with
320 longer duration of action most likely due to rebound effect from
321 hypoglycemia. In contrast to PZI, duration of action of insulin degludec was
322 ≥ 12 h in the most cats irrespective of the doses. These preliminary data
323 justify a study on the use of insulin degludec as once-a-day treatment in
324 diabetic cats.

Another objective of this study was to evaluate the CGSM iPro2 with the Sof-sensor and the Enlite-sensor. From a technical standpoint, the process of initialization of the iPro2, its calibration, and the range of glucose concentrations measured were similar to the real-time CGMS previously validated by our group (Moretti et al., 2009; Moretti et al., 2010; Hafner et al., 2013). Sensor insertion was straightforward, and the implanted sensor did not adversely affect cat's well-being. A unique feature of the CGSM iPro2 is that it allows for continuous glucose monitoring in cats in a home setting, which is a considerable advantage over the currently used real-time CGMSs that require the presence of a digital recorder in close proximity to the sensor. Therefore, the use of iPro2 might be recommended for cats with poor controlled DM, or if stress-induced hyperglycemia, rebound hyperglycemia, or large glycemic variability are suspected.

The Sof-sensor was more reliable with regard to the initial glucose readings; all 20 placed Sof-sensors but only 13/17 Enlite-sensors successfully initiated glucose measurements after placement. Moreover, 17/20 Sof-sensors but only 6/13 Enlite-sensors recorded glucose data during the entire 7-day study period. Of note, 4/13 Enlite-sensors recorded glucose data <50% of the study period. Despite the overall superior performance of the Sof-sensor, the Enlite-sensors had numerically fewer interruptions of <1 h in glucose recording than the Sof-sensors; however, we considered occasional short interruptions in glucose recording of minor importance from a clinical standpoint, because they do not affect the interpretation of glucose curves in a significant way. Interruptions in glucose measurements

349 may occur with either sensor if they are not properly hydrated or regularly
350 calibrated. Even slight changes in the position of the sensor caused by strain
351 on the recorder or the overlying skin can temporarily interrupt
352 measurements. The Enlite-sensor is thinner and shorter than the Sof-sensor
353 and therefore might be more susceptible to interruptions. Taken together,
354 our results suggest that the Sof-sensor is better suited to generate glucose
355 curves in cats than the Enlite-sensor.

356 Both sensors yielded similar and relatively good analytical accuracy based
357 on correlation analysis but did not completely fulfil the ISO criteria. In fact,
358 at glucose concentrations <5.5 mmol/L, both sensors had an analytical
359 accuracy of 90%, which is below the required 95%, and at glucose
360 concentrations ≥ 5.5 mmol/L, the analytical accuracy of the Sof-sensor and
361 Enlite-sensor was 43% and 50%, respectively, both considerably lower than
362 the required 95% accuracy. However, the present study involved healthy
363 cats and focused on hypoglycemia and thus the number of glucose readings
364 ≥ 5.5 mmol/L was low. Including diabetic cats in future studies will aid in
365 evaluation of the analytical accuracy of these sensors for glucose readings
366 ≥ 5.5 mmol/L.

367 Clinical accuracy was evaluated by error grid analysis and was 100% for
368 both sensors in the hypo- and normoglycemic ranges. According to ISO
369 criteria, good clinical accuracy is achieved when at least 99% of glucose
370 measurements fall within zones A and B of the consensus error grid
371 coordinates, which occurred with both sensors in all cats.

372 The study has some limitations. First, it was performed in a small number of
373 healthy young cats, and it might not reflect the effect of tested insulin
374 formulations in older diabetic cats. Sensor glucose readings are affected by
375 the hydration status of the subcutaneous fat, and it might be possible, that
376 the reliability and accuracy of the Sof-sensor and the Enlite-sensor are
377 adversely affected in older diabetic cats with compromised hydration.
378 Second, the method used in the study to assess pharmacodynamic
379 parameters of two insulin formulations is inferior to a isoglycemic clamp
380 method, which has been considered as a gold standard for the study of
381 pharmacodynamics of insulin in people (Heise and Pieber, 2007; Gilor et
382 al., 2010). Traditional blood glucose curves display the effects of exogenous
383 insulin, endogenous insulin, glucagon, and stress hormones. Following
384 severe hypoglycemia, a return of glucose to baseline reflects not only the
385 diminishing effect of exogenous insulin, but could also be the result of
386 activation of glucagon and stress hormones. In few cases (both insulin
387 formulations, data not shown), we recognized a return of glucose
388 concentration to baseline followed by another decline to a smaller degree as
389 before. These could represent counter-regulatory response that masks the
390 effect of exogenous insulin. However, it could also represent physiological
391 glucose fluctuations. It is possible, that pharmacodynamic parameters of
392 both insulin formulations were underestimated due to activation of glucagon
393 and stress hormones. However, we assume, that the counter-regulatory
394 response in cats was similar with both insulin formulations, and
395 pharmacodynamic parameters could be easily compared. Third, we used

396 insulin syringes to inject small amounts of insulin (0.5 – 1.5 IU). Insulin
397 syringes are known to be inaccurate and imprecise at doses lower than 5 IU
398 (Keith et al., 2004).

399 Fourth, the blood glucose was measured with PBGM and not with a routine
400 chemistry analyzer. However, the PBGM AlphaTRAK is specially designed
401 for use in pets. It was evaluated by different research groups, and was
402 shown to be precise and accurate at low, normal and high glucose levels;
403 glucose concentrations measured by AlphaTRAK did not significantly
404 deviate from the reference method (Cohen et al., 2009; Zini et al., 2009).

405 **5. Conclusions**

406 Pritamine zinc insulin and insulin degludec are well tolerated by healthy
407 cats and cause similar degrees of hypoglycemia. Insulin degludec showed
408 longer duration of action than PZI. Further studies on the use of insulin
409 degludec in diabetic cats might be recommended. The CGMS iPro2 with the
410 Sof-sensor or the Enlite-sensor is well tolerated by healthy cats. Both
411 sensors provide good clinical accuracy but analytical accuracy does not
412 reach the minimum set by ISO 15197:2013. The Sof-sensor seems more
413 suitable for use in cats than the Enlite-sensor, because it produces initial
414 glucose readings more reliably and has a better potential to generate glucose
415 curves. However, pharmacodynamics of insulin degludec, and CGMS iPro2
416 need to be investigated in a larger population of diabetic cats.

417

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421

422 **Conflict of interest**

423 Claudia Reusch has a consultancy agreement with Boehringer Ingelheim
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